IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 5386
Gerard *et. al.*	Art Unit: 1652
Application No.: 09/064,057	Examiner: Nashed, N.T.
Filed: April 22, 1998	Atty. Docket: 0942.4330002/RWE/HCC
For: **Recombinant Methods for Making Reverse Transcriptases and Mutants Thereof**	

Declaration Under 37 C.F.R. § 1.132

Attn: AF

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

I, Deb K. Chatterjee, Ph.D., declare as follows:

1. I am presently employed by SAIC-Frederick, National Cancer Institute at Frederick, Maryland. I am a former employee of Invitrogen Corporation, the assignee of U.S. Patent Application No. 09/064,057 ("the '057 application"), filed April 22, 1998, which claims priority to U.S. Patent Application Serial No. 60/049,874, filed June 17, 1997, and U.S. Patent Application Serial No. 60/044,589, filed April 22, 1997. I am also a named inventor in the '057 application.

2. I have conducted and/or directed laboratory research involving reverse transcription of nucleic acid molecules and have engaged in the production of reverse transcriptase by recombinant DNA methods for more than 15 years. A copy of my curriculum vitae is attached as **Exhibit A**.

3. I have read and understand the '057 application. I have also reviewed pending independent claim 26. In addition, I have reviewed Stewart *et al.*, Journal of

Virology 67:7582-7596 (1993), which I understand to be of record in the '057 application as Document No. AT 23.

4. It is my opinion that a "person of ordinary skill in the art" in the technology areas relevant to the '057 application is an individual who has at least an educational background equivalent to a Ph.D. in Molecular Biology (or a related Biological or Chemical field) and at least 2 years of post-doctoral experience conducting laboratory research involving reverse transcription.

5. It is my understanding that the presently claimed invention in the '057 application is directed to a method of producing avian myeloblastosis virus (AMV) reverse transcriptase via the co-expression of an α-subunit of AMV reverse transcriptase and an β-subunit of AMV reverse transcriptase, together, in a eukaryotic cell, and isolating or purifying the expressed AMV αβ reverse transcriptase, such that the resulting AMV αβ reverse transcriptase has a specific activity of at least about 30,000 units per milligram of protein.

6. At Table 7 on page 102 and at page 104, line 25-page 105, line 11, the '057 application states that:

> A comparison was made of the ability of four different forms of RSV RT (αβ, ββ, α, and βp4βp4) to copy RNA. The RNase H active site of each subunit in these enzymes was mutated to eliminate RNase H activity. Each RT was expressed in cultured insect cells and purified by methods described above and in Example 1. Two RNAs were used for comparison: synthetic homopolymer poly(A) and 7.5-Kb mRNA. With poly(A).oligo(dT) as template-primer, a specific activity was calculated by determining an initial rate of poly(dT) synthesis catalyzed at limiting enzyme concentration, and then normalizing the rate to a given mass (mg) of RT in the reaction. This specific activity simply represents the ability of a given RT to incorporate a single deoxynucleotide with an artificial template, and does not

necessarily represent the ability of the enzyme to copy heteropolymeric RNA. With 7.5-Kb RNA as template, the ability of the RTs to make a full-length copy of a long heteropolymeric RNA was assessed (see Example 3 for details). The results are shown above in Table 7.

Two different forms of αβ were characterized in Table 7. One form was generated as the result of the expression of the RSV RT β gene and subsequent proteolytic processing in host insect cells, and had reduced specific and functional activity. The other form of αβ was generated by co-expression of the RSV RT α and β genes. This form of αβ had a similar specific activity to α, approximately 50,000 units/mg, and had a higher specific activity than either ββ or βp4βp4 (approximately 16,000 units/mg). Comparison of the functional activity of this αβ to other RT forms showed a much more dramatic contrast. RSV αβ RT produced 7, 9 and 15 times more total cDNA per mass of enzyme than ββ, βp4βp4 and α, respectively, from 7.5-Kb RNA. Even greater differences were observed when yield of full length product was assessed: RSV αβ RT produced 21, 13 and 146 times more full length product per mass of enzyme than ββ, βp4βp4 and α, respectively. RSV αβ RT produced by co-expression of the RSV RT αH- and βH- genes is therefore much more efficient in copying mRNA than is any other form of RSV RT prepared by analogous methods.

(Table 7 is attached as **Exhibit B**.)

7. The data in Table 7 of the '057 application (hereinafter, "Table 7") show that the RSV αβ RT produced by the co-expression of the α-subunit and β-subunit genes "produced 7, 9 and 15 times more total cDNA per mass of enzyme than ββ, βp4βp4 and α, respectively, from 7.5-Kb RNA" and "21, 13 and 146 times more full length product per mass of enzyme than ββ, βp4βp4 and α, respectively." Based upon the data in Table 7, together with the results discussed on page 104,

line 25 through page 105, line 11 of the '057 application, it is my opinion that a person of ordinary skill in the art would have concluded, as did the Applicants in the '057 application, that "RSV αβ RT produced by co-expression of the RSV RT αH- and βH- genes is therefore much more efficient in copying mRNA than is any other form of RSV RT prepared by analogous methods."

8. The data in Table 7 establish that both the specific activity and the functional activity of an RSV αβ RT generated by the co-expression of the RSV RT α-subunit and β-subunit genes are greater than that of an RSV αβ RT generated by the expression of the β-subunit alone. Specifically, the RSV αβ RT generated by the co-expression of the α and β genes had a greater than 2 fold increase in specific activity, a greater than 3.5 fold increase in the total mass of reverse transcriptase product, and a greater than 7 fold increase in the mass of full-length reverse transcriptase product as compared to the RSV αβ RT generated by the expression of the β-subunit alone.

9. It is my opinion that the data in Table 7 representing the functional activity of RSV-RT are of even more value to one of ordinary skill in the art than the data in Table 7 representing the specific activity of RSV-RT. One of ordinary skill in the art would have appreciated that, as stated in the '057 application, "specific activity simply represents the ability of a given RT to incorporate a single deoxynucleotide with an artificial template, and does not necessarily represent the ability of the enzyme to copy heteropolymeric RNA." However the functional activity data demonstrate "the ability of the RTs to make a full-length copy of a long heteropolymeric RNA," such as long mRNA. In my experience, the vast majority of scientists who utilize reverse transcriptase enzymes, use the enzymes in combination with heteropolymeric templates (*e.g.* mRNAs) and not with artificial homopolymeric templates. Therefore, it is my opinion that the functional activity data in Table 7 are more indicative of the true reverse transcriptase activity of the enzyme.

10. Based upon my experience, it would have been totally unexpected as of the filing date of the '057 application that RSV αβ RT produced by the co-expression of the

α-subunit and β-subunit genes would have had a higher specific activity than RSV αβ RT produced by expression of the β-subunit alone, as is shown in Table 7. It would also have been completely unexpected that RSV αβ RT produced by the co-expression of the α-subunit and β-subunit genes would have had such a substantially higher functional activity than RSV αβ RT produced by expression of the β-subunit alone, as is shown in Table 7.

11. Table 7 and the above-quoted text refer to data obtained from the production of Rous Sarcoma Virus (RSV) reverse transcriptase. However, it is understood in the relevant scientific communities that results for specific and functional activities of RSV reverse transcriptase are analogous and/or equivalent to results for specific and functional activities of AMV reverse transcriptase. Both AMV-RT and RSV-RT are avian sarcoma-leukosis virus (ASLV) reverse transcriptases. As explained in Prasad *et al.*, *Reverse Transcriptase*, Skalka, A.M., and Goff, S.P., Eds., Cold Spring Harbor, New York; Cold Spring Harbor Press, pp. 135-162 (1993), which I understand to be of record as Document No. AR 13 in the '057 application, the active form of ASLV-RTs is a heterodimer of one shorter RT polypeptide and an incompletely processed RT intermediate, termed the α and β polypeptides, respectively. Thus, the active forms of AMV-RT and RSV-RT share the identical polypeptide subunit components. Because of the shared polypeptide subunit components between AMV-RT and RSV-RT, it is my opinion that one of ordinary skill in the art would have understood that the results for specific and functional activities presented in the '057 application for RSV-RT would have been analogous or identical to the results for specific and functional activities for AMV-RT.

12. Additionally, I have reviewed an Amino Acid Sequence Alignment Chart comparing the amino acid sequences of AMV-RT and RSV-RT (attached as **Exhibit C**), as well as the literature upon which it is based. Based upon my understanding of the literature related to ASLV-RTs, it is my opinion that the chart is an accurate depiction of the amino acid sequences of both AMV-RT and RSV-RT. The data in the Amino Acid Sequence Alignment Chart clearly show a

conservation of over 95 percent of the amino acid sequence between AMV-RT and RSV-RT. Because AMV-RT and RSV-RT are from the same family of retroviral reverse transcriptases (ASLV-RT) and have greater than 95 percent homology at the amino acid level, it is my opinion that one of ordinary skill in the art would understand that the results for specific and functional activities presented in the '057 application for RSV-RT could be extrapolated to, and therefore be representative of, the expected results for specific and functional activities in AMV-RT.

13. I am not aware of the commercial availability of a recombinant AMV reverse transcriptase prior to the filing date of the '057 application.

14. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, of both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Further, declarant sayeth not.

_____ _____

Date Deb K. Chatterjee, Ph.D.

Curriculum Vitae

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Management Leadership

Almost 20 years of experience in directing and supervising various groups of scientists in highly reputed industries. Currently, directing a program to support and develop new technologies to fulfill the mission of National Cancer Institute. Maintain high standards in the planning, design and execution of programs. Responsible for creating an environment for scientific professionals that fosters scientific excellence. Experience in team building and providing leadership. In addition, completed extensive professional Management Course and Leadership Training during my tenure at Life Technologies offered by Zenger-Miller and PACE, respectively.

Technical Leadership

Experience in Protein Expression in prokaryotic and eukaryotic systems, Cell-free protein expression, Protein Purification, Protein Engineering, DNA Polymerases, Reverse Transcriptases, Restriction Enzymes, Enzyme Assays/Kinetics, Molecular Biology, Microbial Genetics and Recombinant DNA Techniques. Proven record of excellent research accomplishments and technology development (**42 manuscripts and 25 issued US patents**—please see below).

Professional Experience

2002- present	Associate Director, National Cancer Institute-SAIC, Protein Expression Laboratory, Frederick, Maryland
2000 – 2002	Director/Senior Research Fellow, R&D, Invitrogen-Life Technologies.
1992 – 2000	Research Fellow, Life Technologies.
1991 – 1992	Principal Scientist, Life Technologies.
1988 – 1991	Group Leader, Life Technologies.
1986 – 1988	Senior Scientist, Life Technologies.
1985 – 1986	Visiting Scientist, EPA, Gulf Breeze, Florida
1979 – 1985	Post-doctoral Fellow, U of Illinois, Chicago.

Awards/Honors

NCI-SAIC Outstanding Scientific Achievement of the year Award (2003)
NCI-SAIC Technology Development Fund Award (2003)
Two consecutive LTI President Awards for excellence in leadership (1998, 1999)
Two Dexter Awards for outstanding Technical Innovations (1992, 1996)
National Merit Scholarship Award, Government of India

Professional duties

Regular reviewer of Journal articles and Research grants.
Invited speaker –Universities, domestic and international conferences

Patents

25 US Patents awarded while at Life Technologies
6 US Patents submitted from Life Technologies/Invitrogen
2 US Patents submitted from NCI-SAIC

List of Publications:

1. Ghosh, J.J., Mitra, G., Poddar, M.K. and Chatterjee, D.K. (1977). Effect of Δ^9-tetrahydrocannabinol administration of hepatic functions. Biochem. Pharmacol. 26, 1797-1801.

2. Guha, M., Chatterjee, D.K., Hati, R. and Datta, A.G. (1978). Existence of an inhibitor of iodination reaction in sheep sub-maxillary extract. J. Ind. Chem. Soc. LV, 103-104.

3. Chatterjee, D.K., Banerjee, R.K. and Datta, A.G. (1980). Studies on peroxidase catalysed formation of thyroid hormones on a protein isolated from sub-maxillary gland. Biochem. Biophys. Acta. 612, 29-39.

4. Chatterjee, D.K., Kellogg, S.T., Hamada, S. and Chakrabarty, A.M. (1981). Plasmid specifying total degradation of 3-chlorobenzoate by a modified *ortho* pathway. J. Bacteriol. 146, 639-646.

5. Chatterjee, D.K., Kellogg, S.T., Watkins, D.R. and Chakrabarty, A.M. (1981). Plasmids in the Biodegradation of Chlorinated Aromatic Compounds. In: *Molecular Biology, Pathogenicity and Ecology of Bacterial Plasmids*, S. B. Levy, Royston C. Clowes and E. Koenig, Eds., Plenum Publishing Corp., New York, 519-528.

6. Kellogg, S.T., Chatterjee, D.K. and Chakrabarty, A.M. (1981). Plasmid assisted molecular breeding - New technique for enhanced biodegradation of persistent toxic chemicals. Science, 214, 1133-1135.

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10. Karns, J.S., Kilbane, J.J., Chatterjee, D.K. and Chakrabarty, A.M. (1981). Laboratory Breeding of a Bacterium for Enhanced Degradation of 2,4,5-T. In: *Genetic Engineering for Biotechnology*, J. Cromoco, Ed., Promocet, Sao Paulo, Brazil, 37-40.

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12. Chatterjee, D.K., Kilbane, J.J. and Chakrabarty, A.M. (1982). Biodegradation of 2,4,5-T in soil by a pure culture of *Pseudomonas cepacia*. Appl. Environ. Microbiol., 44, 514-516.

13. Chatterjee, D.K. and Chakrabarty, A.M. (1982). Genetic rearrangements in plasmids specifying total degradation of chlorinated benzoic acids. Mol. Gen. Genet., 188, 279-285.

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References: Available upon request

Table 7. ***Specific and Functional Activities of Various Forms of RSV RTs Expressed in Insect Cells***

Gene(s) Expressed	RT Form Isolated											
	RSV αβ RT			RSV ββ RT			RSV α RT			RSV βp4βp4 RT		
	Specific Activity[a] (U/mg)	Functional Activity[b]		Specific Activity (U/mg)	Functional Activity		Specific Activity (U/mg)	Functional Activity		Spec. Act. (U/mg)	Functional Activity	
		Total (ng/μg)[c]	Full-length (ng/μg)[d]		Total (ng/μg)	Full-length (ng/μg)		Total (ng/μg)	Full-length (ng/μg)		Total (ng/μg)	Full-length (ng/μg)
α and β	53,191	4,092	874	NP[e]	NP	NP	ND[f]	ND	ND	NP	NP	NP
β	25,113	1,098	116	15,819	584	41	ND	ND	ND	NP	NP	NP
βp4	NP	NP	NP	NP	NP	NP	NP	NP	NP	15,984	450	67
α	NP	NP	NP	NP	NP	NP	48,759	272	6	NP	NP	NP

[a]Specific activity = units (U) RT activity /mg RT protein in poly(A)•oligo(dT) assay (Houts *et al., J. Virol. 29*:517 (1979)).

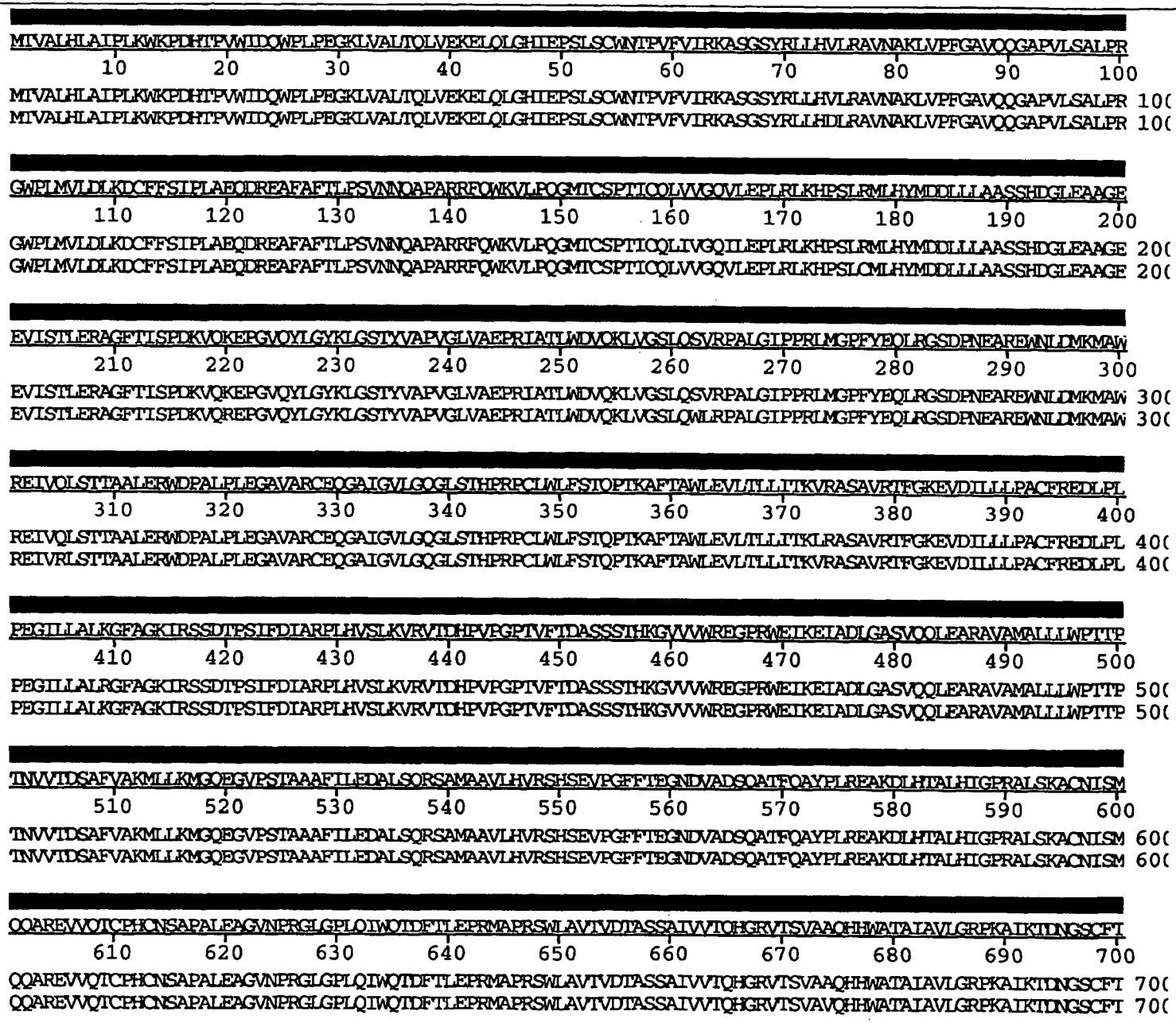
[b]Functional activity established with 7.5 Kb RNA as described in Example 3.

[c]Mass of total reverse transcribed product, ng of product produced per μg of RT used.

[d]Mass of full-length reverse transcribed product, ng of product produced per μg of RT used.

[e]"NP" = RT form not produced by insect cells expressing the indicated gene.

[f]"ND" = RT form produced by insect cells, but not analyzed in present studies.


10 20 30 40 50 60 70 80 90 100
amv-rt.pro MTVALHLAIPLKWKPDHTPVWIDQWPLPEGKLVALTQLVEKELQLGHIEPSLSCWNTPVFVIRKASGSYRLLHVLRAVNAKLVPFGAVQQGAPVLSALPR 10(
rsv-rt.pro MTVALHLAIPLKWKPDHTPVWIDQWPLPEGKLVALTQLVEKELQLGHIEPSLSCWNTPVFVIRKASGSYRLLHDLRAVNAKLVPFGAVQQGAPVLSALPR 10(
110 120 130 140 150 160 170 180 190 200
amv-rt.pro GWPLMVLDLKDCFFSIPLAEQDREAFAFTLPSVNNQAPARRFQWKVLPQGMTCSPTICQLIVGQILEPLRLKHPSLRMLHYMDDLLLAASSHDGLEAAGE 20(
rsv-rt.pro GWPLMVLDLKDCFFSIPLAEQDREAFAFTLPSVNNQAPARRFQWKVLPQGMTCSPTICQLVVGQVLEPLRLKHPSLCMLHYMDDLLLAASSHDGLEAAGE 20(
210 220 230 240 250 260 270 280 290 300
amv-rt.pro EVISTLERAGFTISPDKVQKEPGVQYLGYKLGSTYVAPVGLVAEPRIATLWDVQKLVGSLQSVRPALGIPPRLMGPFYEQLRGSDPNEAREWNLDMKMAW 30(
rsv-rt.pro EVISTLERAGFTISPDKVQREPGVQYLGYKLGSTYVAPVGLVAEPRIATLWDVQKLVGSLQWLRPALGIPPRLMGPFYEQLRGSDPNEAREWNLDMKMAW 30(
310 320 330 340 350 360 370 380 390 400
amv-rt.pro REIVQLSTTAALERWDPALPLEGAVARCEQGAIGVLGQGLSTHPRPCLWLFSTQPTKAFTAWLEVLTLLITKLRASAVRTFGKEVDILLLPACFREDLPL 40(
rsv-rt.pro REIVRLSTTAALERWDPALPLEGAVARCEQGAIGVLGQGLSTHPRPCLWLFSTQPTKAFTAWLEVLTLLITKVRASAVRTFGKEVDILLLPACFREDLPL 40(
410 420 430 440 450 460 470 480 490 500
amv-rt.pro PEGILLALRGFAGKIRSSDTPSIFDIARPLHVSLKVRVIDHPVPGPTVFTDASSSIHKGVVVWREGPRWEIKEIADLGASVQQLEARAVAMALLIWPTTP 50(
rsv-rt.pro PEGILLALKGFAGKIRSSDTPSIFDIARPLHVSLKVRVIDHPVPGPTVFTDASSSIHKGVVVWREGPRWEIKEIADLGASVQQLEARAVAMALLIWPTTP 50(
510 520 530 540 550 560 570 580 590 600
amv-rt.pro TNVVIDSAFVAKMLLKMGQEGVPSTAAAFILEDALSQRSAMAAVLHVRSHSEVPGFFTEGNDVADSQATFQAYPLREAKDLHTALHIGPRALSKACNISM 60(
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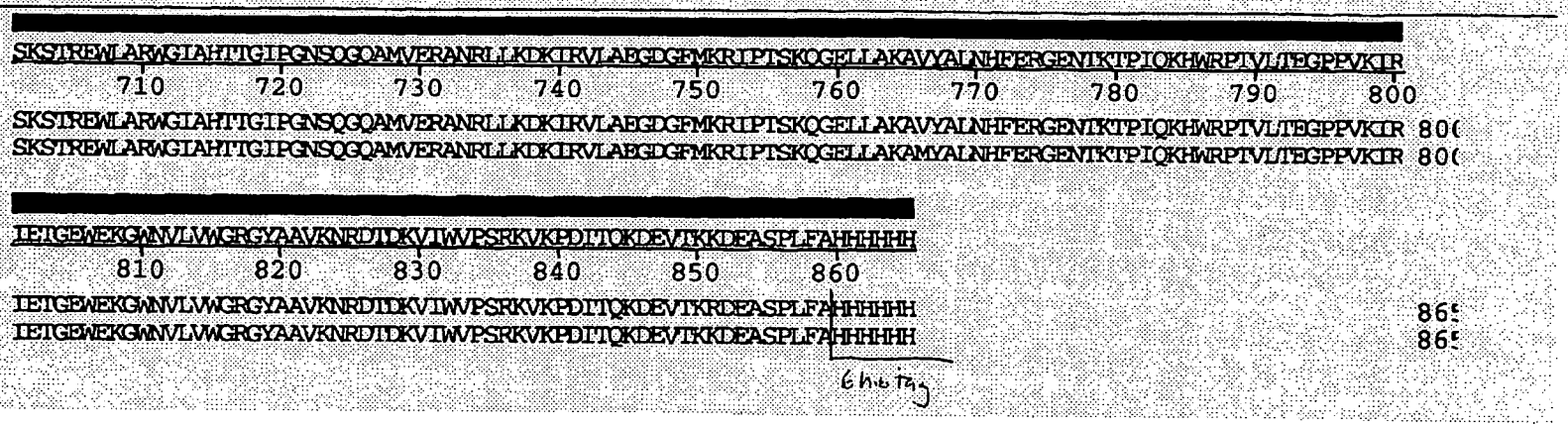
rsv-rt.pro QQAREVVQTCPHCNSAPALEAGVNPRGLGPLQIWQTDFTLEPRMAPRSWLAVTVDTASSAIVVTQHGRVTSVAVQHHWATAIAVLGRPKAIKTDNGSCFT 70(


710
720
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amv-rt.pro
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